

CLAIMS:

1. A process for producing enzyme particles, which process includes

5 providing an emulsion of droplets of a first liquid phase dispersed in a second liquid phase, with the one liquid phase being a hydrophilic phase and the other liquid phase being a hydrophobic phase which is immiscible with the hydrophilic phase, and with enzyme molecules being located at or within interfacial boundaries of the droplets and the second liquid phase;

10 cross-linking the enzyme molecules of the respective droplets so that individual enzyme particles, which are stable and in which the enzymes are immobilized with a majority of active sites of the enzymes being orientated either internally or externally, are formed from individual droplets; and

15 recovering the individual enzyme particles from the second liquid phase.

2. A process according to Claim 1, wherein the individual particles have openings so that the liquid phases can pass in or out of the particles.

20 3. A process according to Claim 1, wherein individual particles are liquid impervious.

4. A process according to any one of Claims 1 to 3 inclusive, which includes adding to the hydrophilic phase and/or to the hydrophobic phase 25 and/or to the emulsion, a modifier for modifying the hydrophobicity and/or charge of the enzyme.

5. A process according to any one of Claims 1 to 4 inclusive, wherein the enzyme is a lipase.

30 6. A process according to Claim 5, wherein the lipase is selected from *Pseudomonas cepacia* lipase, *Pseudomonas fluorescens* lipase, *Pseudomonas alcaligenes* lipase, *Candida rugosa* lipase, *Candida antarctica* lipase A, *Candida antarctica* lipase B, *Candida utilis* lipase, *Thermomyces*

lanuginosus lipase, Rhizomucor miehei lipase, Aspergillus niger lipase, Aspergillus oryzae lipase, Penicillium sp lipase, Mucor javanicus lipase, Mucor miehei lipase, Rhizopus arrhizus lipase, Rhizopus delemere lipase, Rhizopus japonicus lipase, Rhizopus niveus lipase, and Porcine Pancreatic lipase.

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7. A process according to Claim 5 or Claim 6, wherein the provision of the emulsion is effected by dissolving the enzyme in the hydrophilic or W phase and forming the emulsion by mixing the enzyme containing hydrophilic phase with the hydrophobic or O phase.

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8. A process according to Claim 7, which includes selectively precipitating the enzyme at the interface when the emulsion is a O/W emulsion in which hydrophobic phase droplets are dispersed in a continuous hydrophilic phase, or within the droplet volume, when the emulsion is a W/O emulsion in which hydrophilic phase droplets are dispersed in a continuous hydrophobic phase.

9. A process according to Claim 7 or Claim 8, wherein the cross-linking of the enzyme molecules is effected by means of a cross-linking agent which is added to the hydrophilic phase and/or to the hydrophobic phase and/or to the emulsion.

10. A process according to Claim 9, which includes adding to the hydrophilic phase and/or to the hydrophobic phase and/or to the emulsion, a temporary protectant that occupies active sites of the enzyme during the cross-linking, thereby inhibiting occupation of or reaction with the active sites by the cross-linking agent.

11. A process according to any one of Claims 7 to 10 inclusive, which includes adding an amino acid to the emulsion to inhibit agglomeration of the individual enzyme particles.

12. A process according to any one of Claims 7 to 11 inclusive, which includes recovering the enzyme particles from the second liquid phase.

13. A process according to any one of Claims 7 to 12 inclusive, which includes extracting the first liquid phase from the enzyme particles.

5 14. A process according to any one of Claims 7 to 13 inclusive, wherein the hydrophilic phase comprises water and, optionally, a buffer in the water.

10 15. A process according to any one of Claims 7 to 13 inclusive, wherein the hydrophilic phase comprises a polyethylene glycol and, optionally, water admixed with the polyethylene glycol.

15 16. A process according to any one of Claims 7 to 15 inclusive, wherein the hydrophobic phase comprises an oil; a hydrocarbon; an ether; or an ester.

20 17. A process according to any one of Claims 7 to 16 inclusive, wherein the emulsion is a W/O emulsion in which hydrophilic phase droplets are dispersed in a continuous hydrophobic phase, with a second enzyme, co factor and/or mediator being present in the hydrophilic phase.

25 18. A process according to any one of Claims 5 to 16 inclusive, wherein a triglyceride, which is hydrolysable by lipase, is used as the hydrophobic phase, with an O/W emulsion, in which hydrophobic phase droplets are dispersed in a continuous hydrophilic phase, being formed and with the dispersed hydrophobic phase contained within the cross-linked particles being hydrolyzed by the lipase during and after the cross-linking reaction.

30 19. A process according to any one of Claims 7 to 16 inclusive, wherein an initial O/W emulsion, in which hydrophobic phase droplets are dispersed in a continuous hydrophilic phase, is formed, with the process including, before effecting the cross-linking, centrifuging the emulsion and separating a concentrated emulsion from a dilute hydrophilic phase, to

increase lipase purity; and inverting the emulsion to form a W/O emulsion in which hydrophilic phase droplets are dispersed in a continuous hydrophobic phase, by the addition of a surfactant with a lower HLB value.

- 5 20. A process according to any one of Claims 1 to 19 inclusive wherein, to impart specific properties to the enzyme particles, a modifier is added to the hydrophilic phase and/or to the hydrophobic phase and/or to the emulsion.
- 10 21. A process according to Claim 20, wherein the modifier is a surfactant, for imparting enhanced enzyme activity and improved emulsion stability.
- 15 22. A process according to Claim 20, wherein the modifier is a precipitator for precipitating the enzyme onto the emulsion interfaces.
- 20 23. A process according to Claim 20, wherein the modifier is an additive for modifying the pH; ionic strength; viscosity; magnetic properties; agglomeration tendency; and/or zeta potential of the emulsion and/or the enzyme particles.
- 25 24. An enzyme particle, which comprises cross-linked enzyme molecules so that the particle is stable, with the particle being hollow, and in which the enzymes are immobilized, with a majority of active sites of the enzymes being orientated either internally or externally.
- 25 25. An enzyme particle according to Claim 24, which is spherical.
- 30 26. An enzyme particle according to Claim 24 or Claim 25, which contains, in its lumen, a liquid.
27. An enzyme particle according to any one of Claims 24 to 26 inclusive, wherein the enzyme is a lipase.

28. An enzyme particle according to Claim 27, wherein the lipase is selected from *Pseudomonas cepacia* lipase, *Pseudomonas fluorescens* lipase, *Pseudomonas alcaligenes* lipase, *Candida rugosa* lipase, *Candida antarctica* lipase A, *Candida antarctica* lipase B, *Candida utilis* lipase,

5 *Thermomyces lanuginosus* lipase, *Rhizomucor miehei* lipase, *Aspergillus niger* lipase, *Aspergillus oryzae* lipase, *Penicillium* sp lipase, *Mucor javanicus* lipase, *Mucor miehei* lipase, *Rhizopus arrhizus* lipase, *Rhizopus delemere* lipase, *Rhizopus japonicus* lipase, *Rhizopus niveus* lipase, and *Porcine Pancreatic* lipase.

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29. A method of carrying out a reaction, which includes allowing a reaction medium to undergo a reaction in the presence of a plurality of the enzyme particles according to any one of Claims 24 to 28 inclusive, with the reaction thus being catalyzed by the enzyme particles.

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